plication induced marked channel activation. In symmetrical 210mM K^+ and 10μM Ca^{2+}, NAADP dose-dependently activated TPC2 channels with an EC_{50} of 500nM. Addition of 200μM *trans* Ca^{2+} significantly increased the sensitivity of TPC2, shifting the EC_{50} to 5nM. We have previously demonstrated that ligand-activation of RyR channels is also highly sensitive to luminal Ca^{2+} and therefore we have investigated how NAADP affects RyR1 and RyR2 in the presence of sensitising levels of luminal Ca^{2+}. Addition of NAADP ($\leq$1μM) did not affect RyR2 Po but slightly activated RyR1 (1μM NAADP increased Po from 0.022 ± 0.035 to 0.106 ± 0.147; SD, n=5). In contrast, larger increases in TPC2 Po (0.001 ± 0.002 to 0.4 ± 0.2; SD, n=3, $P<0.05$) could be elicited with much lower NAADP concentrations (10nM). Our study is the first to show that animal TPCs form functional, Ca^{2+}-permeable ion-channels. We also provide further evidence that TPC2 is capable of mediating NAADP-sensitive Ca^{2+}-release from acidic organelles but do not rule out a role for RyR1.
BHF supported

Membrane Transporters & Exchangers II

3559-Pos
Functional Reconstitution of Influenza a M2 (22-62)
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Amantadine-sensitive selective proton uptake by liposomes is currently the ideal method of demonstrating M2 functionality after reconstitution, validating structural determination of the reconstituted protein such as that carried out using solid state NMR (e.g. with M2 22-62, Sharma et al, this meeting). Under pH and Vm gradients, the truncated construct (which lacked 21 residues at the N-terminus and 35 at the C-terminus) was shown to transport protons at the same rate (21 H+/s at pH 6.5) as a similar construct, M2 (18-60), which elsewhere had been shown to transport similarly to other variants, including the full length protein. 100 μM amantadine was found to reduce transport by ~80%, and 10 μM amantadine or cyclooctylamine reduced transport by 50%. Transport was optimal at protein densities of 0.05-1.0% (weight peptide of weight protein and lipid). At 10%, transport was reduced, presumably due to density-dependent ion leakage. Reduction of pH to 5.0 increased transport. Rundown of total proton uptake after addition of valinomycin and CCCP, as detected by delaying application of valinomycin, indicate M2-induced K+ flux of <1 K+/s and that permeability (flux/concentration) of M2 22-62 to K+, relative to H+, is <10-7. Transport rate, amantadine and cyclooctylamine sensitivity, acid activation, and H+ selectivity are all consistent with full functionality of the reconstituted protein construct.

3560-Pos
***Yersinia* Translocon Complexes are Stabilized in Nanolipoprotein Particles (NLPs)**
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To enter host cells and evade host defenses, many gram-negative bacteria, including the plague pathogen, *Yersinia pestis*, utilize proteins that are able to interact with and enter host membranes. One particular mechanism for invasion is the type III secretion system, which is a highly ordered complex for injecting bacterial proteins into host cells, using a complex referred to as a translocon pore. Our results show cell-free expression of YopB and YopD was enhanced in the presence of liposomes or NLPs. However, liposomes containing YopB/D tended to aggregate and precipitate. In order to enable the study of the type III secretion proteins we have applied cell-free approaches for producing soluble *Y. pestis* membrane associated proteins YopB and YopD that are involved in the translocon pore as a complex supported by nanolipoprotein particles (NLPs). With addition of NLP, the YopB/D complex was rendered soluble. AFM showed that soluble YopB/D complex was associated with NLPs as measured by a height increase compared to NLPs not containing YopB/D. Preliminary AFM results also demonstrated binding between LcrV and YopB/D-NLPs which is indicative of proper folding in the NLP structure. Interaction studies of the YopB/D translocon embedded in a membrane with effectors such as LcrV may elucidate the poorly understood pore-forming event that helps this pathogen to evade the host defenses. Our method is applicable to other membrane proteins and represents a unique solution to solubility and purification problems.

3561-Pos
Location of Transmembrane Segments of Na^+/ca^{2+} Exchanger NCX1 Investigated with Chemical Crosslinkers
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The sodium-calcium exchanger (NCX1) is a plasma membrane protein important in regulating calcium in cardiac myocytes. The topological model is comprised of nine transmembrane segments (TMs) and a large intracellular loop, which has two Ca^{2+} binding domains (CBD1 and CBD2), between TMs five and six. CBD1 and CBD2 have been crystallized recently and are important in regulating the function of NCX1. On the other hand, the three dimensional structure of the full length NCX1 is unknown. To gain insights into that 3-D structure, we performed cysteine crosslinking experiments. Pairs of amino acids in different TMs were mutated to cysteine on the backbone of cysteine-less NCX1. The mutated NCXs were expressed in an insect cell line and treated with cysteine-specific chemical crosslinkers followed by SDS-PAGE to determine the proximity of the introduced cysteines. The results allow us to place TMS I, IV and IX into the context of the other TMS. By combining our new results with our previous work (*J Biol Chem. 2006, 281: 22808-14; J Biol Chem. 2001, 276:194-9.*) , we propose that TMs II and VII, which contain a number of hydrophilic residues, are surrounded by the remaining TMs.

3562-Pos
Ca^{2+}-Induced Conformational Changes of Na^+-Ca^{2+} Exchanger Dimers: Role of Ca^{2+} Binding Domains
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The Na^+-Ca^{2+} exchanger is activated by the binding of cytoplasmic Ca^{2+} to two Ca^{2+} binding domains (CBD1 and CBD2). How binding of Ca^{2+} is translated into exchanger activation is unknown. We investigated Ca^{2+}-dependent movements as changes in FRET between exchanger dimers tagged with CFP or YFP at positions 266 within the large cytoplasmic loop of NCX1.1. The biophysical properties of the fluorescent exchangers are identical to those of the untagged NCX. Fluorescent exchangers were coexpressed in Xenopus oocytes from which plasma membrane sheets were isolated. Upon addition of Ca^{2+}, the coexpressed pair NCX-266CFP + NCX-266YFP showed an increase in FRET in a dose-dependent manner. Similar FRET changes were observed after mutating the Ca^{2+} coordination site in CBD2 (E516L). Exchanger E516L is not Ca^{2+} regulated. In contrast, mutating the Ca^{2+} coordination site in CBD1 (D421A, E451A and D500V) abolished FRET changes. These residues likely disrupt binding of Ca^{2+} to CBD1. Nevertheless, Ca^{2+} regulation of NCX is retained though with a substantial decrease in apparent affinity for Ca^{2+}. These results indicate that the Ca^{2+}-induced conformational changes of NCX dimers arise exclusively from the movement of CBD1. Peptides of Ca^{2+} binding domains, flanked by CFP and YFP, recapitulated the full length exchanger results: CBD1 showed movement upon Ca^{2+} addition while CBD2 did not. A peptide spanning CBD1-CBD2 displayed Ca^{2+}-dependent movement, which was abolished by mutating the Ca^{2+} coordination site in CBD1. Our results indicate the following: 1. Exchanger conformational changes are associated with the occupancy of a high affinity Ca^{2+} binding site exclusively within CBD1. 2. FRET studies confirm that the Na^+-Ca^{2+} exchanger exists as a dimer.

3563-Pos
The Role of Microscopic Interactions for Effective Antibiotic Delivery across the Bacterial Outer Membrane
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Outer membrane protein F (OmpF) allows diffusion of beta-lactam antibiotics across the outer membrane of Gram-negative bacteria. The diffusion limit for translocating molecules is provided by the constriction zone, which defines both the channel diameter at the narrowest region, as well as electrostatic properties due to a unique arrangement of charged residues. Since reduced outer membrane permeability contributes to antimicrobial resistance, it is necessary to identify the role of drug-protein molecular interactions in antibiotic transfer in order to design antibiotics with improved diffusional characteristics. We have co-crystallized *E. coli* OmpF with various antibiotic molecules and observe the density corresponding to the antibiotic inside the OmpF pore. Results of this work give insights into how the charge distribution of the translocating molecule affects binding interactions within the OmpF constriction zone. Furthermore, functional assays and mutational analysis provide evidence that alteration of some key charged OmpF residues has an effect on bacterial cell survival. We are also using computational methods to model the pathways of diffusing antibiotics and measure their residence time in the OmpF pore.